Morphology of the Ear of the Shark Genus *Carcharhinus*, with Particular Reference to the Macula Neglecta[1]

ALBERT L. TESTER,[2] JAMES I. KENDALL,[2] AND WILLIAM B. MILISEN[2]

OUR INTEREST in the morphology of the so-called "macula neglecta" in sharks was stimulated by Lowenstein's and Roberts's (1951) electrophysiological demonstration that, in the ray, this little-known sensory epithelium of the ear is a remarkably sensitive vibration receptor. As made clear by Lowenstein and Roberts, however, the question remains as to whether this macula or the adjacent macula of the sacculus can mediate water-borne sounds. Sound is propagated both as displacement (particle velocity) waves and as pressure waves (Van Bergeijk, 1967), the latter requiring some transducing mechanism for converting pressure to displacement in order to be "heard" by the aquatic animal.

According to de Burlet (1934), the macula neglecta was first discovered in the utriculus of bony fishes by Retzius (1881, 1884) and was so named because it somewhat resembled other maculae of the ear and had been overlooked until then. De Burlet stated that the macula neglecta has also been called the crista neglecta and crista quarta, although he gave it the "neutral" designation of papilla neglecta. Since in sharks this structure does not resemble a papilla we will continue to call it the macula neglecta, even though it may have a cupula rather than an otolith associated with its sensory epithelium.

De Burlet (1934) pointed out that the macula neglecta appears to occur in all classes of vertebrates, although rarely in amphibians and mammals. It is generally present in fishes. In bony fishes it lies in the utriculus near the opening to the ampulla of the posterior vertical canal. He stated that in selachians it lies within a duct through which the posterior vertical canal connects with the sacculus. Quiring (1930) stated that in *Acanthias vulgaris* the hood-shaped "macula acoustica neglecta" lies at the entrance to the posterior vertical canal, projecting slightly into the cavity of the sacculus. According to the diagrams of Lowenstein and Roberts (1951), in the ray the macula neglecta lies in the wall of the sacculus adjacent to the opening of the duct.

Lowenstein and Roberts (1951) also pointed out the proximity of the macula neglecta to the membrane-covered "fenestra ovalis," an oval perforation of the cartilaginous skull, first described by Scarpa in 1789 (Ayers, 1892). They suggested that this structure, together with the adjoining ductus endolymphaticus, could represent a sound-conducting mechanism aimed at the sacculus macula and the macula neglecta. Historically, it might be pointed out that Howes (1883) advanced the theory that the membrane of the fenestra is the first trace of a vertebrate tympanum but that this presumed homology was refuted by Ayers (1892) on the basis of different ontogenetic derivation of the two structures.

In the present paper we give a brief general description of the ear of *Carcharhinus*, hitherto undescribed, but concentrate on the morphology, histology, and innervation of the macula neglecta and its relationship with the fenestra ovalis, posterior canal duct, sacculus, and endolymphatic duct. It is hoped that the information will be useful to behaviorists and electrophysiologists in further experimentation aimed at clarifying the role of the ear in elasmobranch hearing.

MATERIALS AND METHODS

Gross dissection was performed on several juvenile and/or adult specimens (100 to 215 cm total length) of each of the following species: gray reef shark, *Carcharhinus menisorrah* (= *C. amblyrhynchos*, J. A. F. Garrick, personal communication) from Eniwetok Atoll and Hawaii; blacktip reef shark, *C. melanop-*

[1] Manuscript received 10 December 1971.

[2] University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

terus, from Christmas Island; sandbar shark, *C. milberti,* from Hawaii; and blacktip shark, *C. limbatus,* from Hawaii. Effort was concentrated on the gray reef shark which seemed typical of the genus.

After the endolymphatic pores were located, the integument removed, and the endolymphatic ducts examined, the portion of the chondrocranium containing the otic capsules was removed with a sharp, heavy knife from fresh or fresh-frozen heads. Dissection then proceeded either on the fresh or thawed material or after preservation of the capsules in 10-percent formalin. The cartilage was removed from the labyrinth by careful slicing with knife or scalpel, or in calcified areas by crushing with long-nosed pliers and picking away the pieces. Chlorozal Black or Giemsa stain introduced by hypodermic needle into the endolymph of the canals and chambers made them more readily visible.

Both whole mounts and frozen sections were made of various parts of the labyrinth to study the shape, position, and innervation of the various maculae and cristae. They were generally stained with methylene blue or Giemsa for epithelial tissue and with silver nitrate (Gilbert's technique) for nerves, and cleared in xylol.

The otic capsules of several unborn *C. menisorrah* pups, 25 to 35 cm total length, were preserved in either 10 percent formalin or Bouin's fixative and were cut according to standard paraffin sectioning technique. The few calcified areas were readily decalcified by the Bouin's fluid. Series of sections were cut at 10 to 15 μ vertically, horizontally, and transversely through the ear, and also obliquely along the axis of the macula neglecta. They were stained for general tissue examination by Mallory's triple, hematoxylin-eosin, or hematoxylin fast green, for mucopolysaccharides by Giemsa or Gomori-aldehyde-fuchsin, and for nerve fibers by the 2-hour silver technique of Davenport (1961) followed by gold toning.

To visualize better the complex system of canals and sacs comprising the labyrinth, a 3-dimensional model was constructed based on information obtained from dissections and sections. This was first molded freehand in potter's clay and then reproduced in plaster of Paris, using a supporting structure of galvanized steel wire with soldered joints, overlaid with strips of gauze soaked in plaster. The final replica was made by troweling on plaster to achieve proper dimensions, sanding to shape, coating with latex paint, and mounting with two bronze rods on a resin base.

RESULTS

Medial and lateral views of the left labyrinth of *Carcharhinus menisorrah,* photographed from the model, are shown in Fig. 1 and 2. The connections of the various ducts and sacs are shown schematically in Fig. 3. In general, the structure of the ear is similar to that of other elasmobranchs and will not be described in detail except for those parts that may relate to the functioning of the macula neglecta. In contrast to bony fishes, each labyrinth opens to the exterior via an endolymphatic duct arising from the apex of the sacculus, and the posterior vertical canal connects with the sacculus, rather than with the utriculus, via a connecting tube, the posterior canal duct (cf. Davies, Lochner, and Smith, 1963).

Endolymphatic Ducts

The external pores of the endolymphatic ducts, bordered by scales, are oval or rectangular in shape with an aperture of about 0.3 by 0.5 mm in a 160-cm *Carcharhinus.* They are located on the dorsal surface of the head just anterior to the supratemporal lateralis canal and are separated by a distance of about 3 cm. The ducts lie in the parietal fossa, a dished-out area on the dorsal surface of the chondrocranium. Each duct leads from its pore in an anteromedial direction, sloping slightly ventrally until the two ducts meet at a point approximately in the center of the fossa. Although the walls of the ducts meet, their lumens do not join; each lumen remains separate as the ducts double back on themselves ventrally and extend a short distance posteriorly before separating, each turning laterally to penetrate the chondrocranium. The lumen of the duct has a maximum diameter of about 2 mm in a 160-cm *Carcharhinus;* it is not nearly as enlarged as in the dogfish or ray in which it expands into a pouch or sac before entering the chondrocranium.

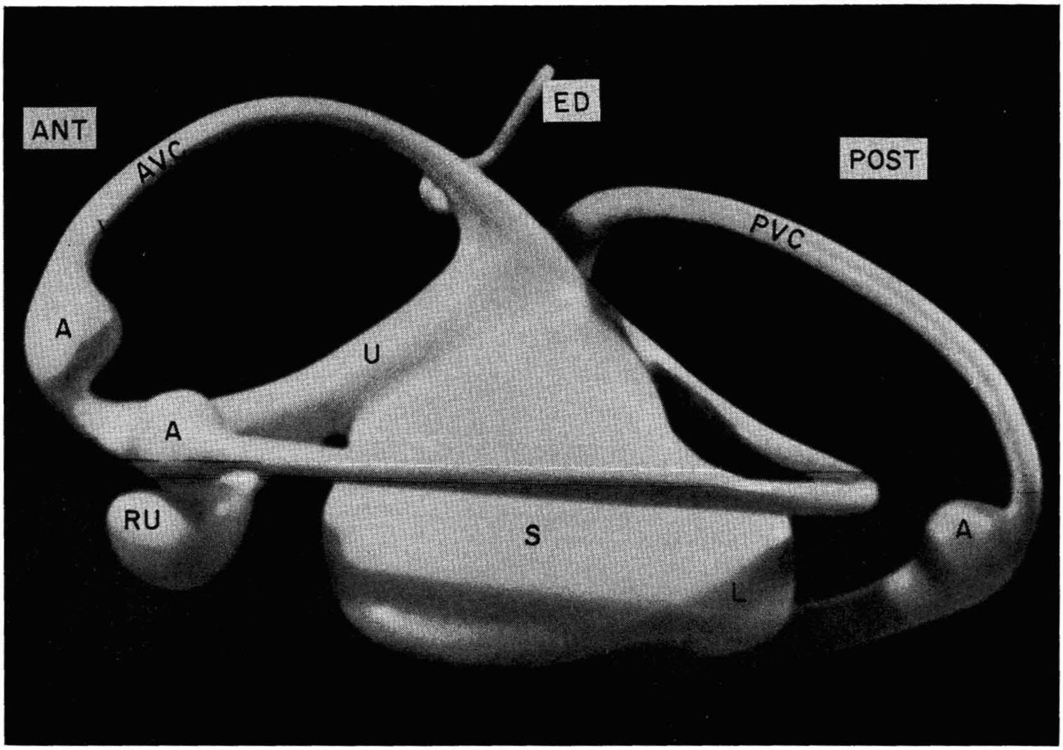

FIG. 1. Lateral view of the left ear of *Carcharhinus menisorrah*, photographed from a model. *A*, ampulla; *ANT*, anterior; *AVC*, anterior vertical canal; *ED*, endolymphatic duct; *L*, lagena; *POST*, posterior; *PVC*, posterior vertical canal; *RU*, recessus utriculi; *S*, sacculus; *U*, utriculus.

The lumen of the endolymphatic duct is usually packed with white or brownish, opaque granules called otoconia or statoconia (Fig. 4), which effervesce and dissolve in dilute HCl and presumably are composed of calcium carbonate in the form of aragonite (Carlström, 1963). The smallest granules are round to oval spherules, about 2 μ in diameter, and sometimes show Brownian movement in a water mount. Often several small granules are encased in a mucous sheath. They are the most numerous of the various sized groups. The larger granules become elongated, have rounded ends, and are shaped like an American football. They range from about 3 by 4 μ to about 70 by 125 μ in diameter, decreasing exponentially in relative numbers with increase in size. The size range is somewhat greater than that reported by Carlström (1963) for carcharhinid and other sharks ("a few to about 40 microns"). Occasionally the granules have radial etchings on their surface. Herzog (1925) found that these granules, when stained with hematoxylin, often appear to have a "nucleus." In addition, we found that stained granules sometimes show concentric layers suggesting stages of growth. A few of the granules are irregularly shaped because of the fusion of two or more small particles. In one specimen we found a few pyramid-shaped granules similar to those described by Carlström (1963) in *Galeocerdo cuvieri* and identified by him as the rare calcium carbonate monohydrate; however, in our specimen these granules may have been formed after death as a result of poor preservation of the frozen material.

Calcium carbonate granules were not observed in sections of the pup endolymphatic duct.

In both pup and adult, the epithelial lining of the endolymphatic duct consists of columnar cells, the bulbous ends of which project into the lumen and stain metachromatically with Giemsa and Gomori-aldehyde-fuchsin. This is

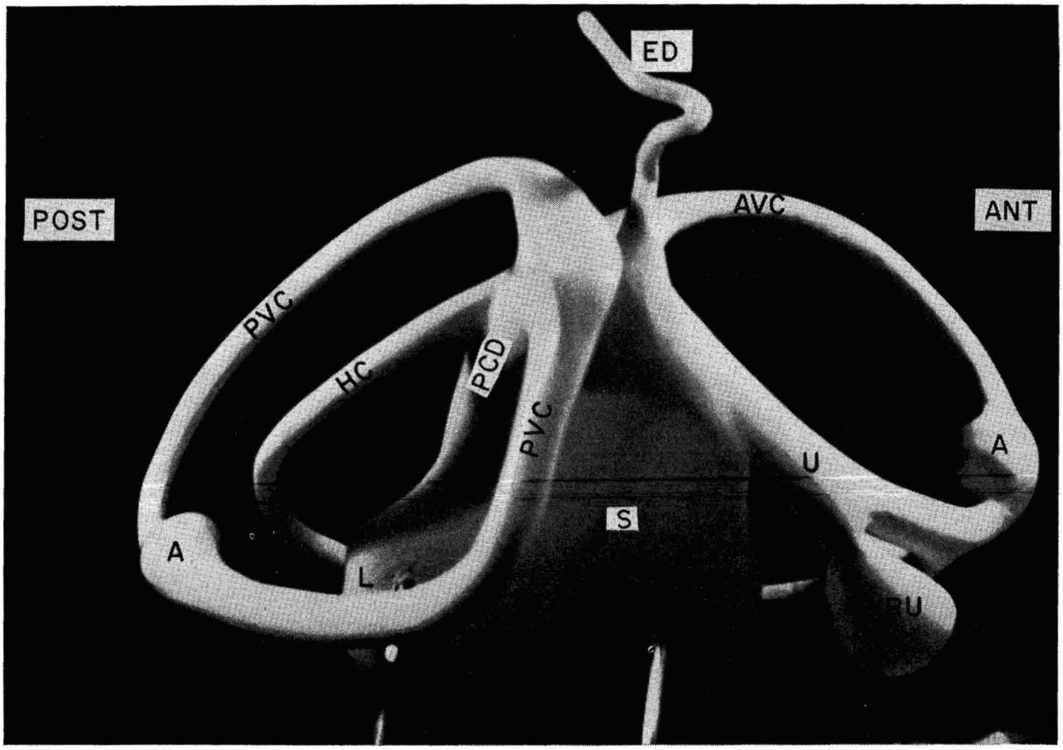

FIG. 2. Medial view of the left ear of *Carcharhinus menisorrah*, photographed from a model. *A*, ampulla; *ANT*, anterior; *AVC*, anterior vertical canal; *ED*, endolymphatic duct; *HC*, horizontal canal; *L*, Lagena; *POST*, posterior; *PCD*, posterior canal duct; *PVC*, posterior vertical canal; *RU*, recessus utriculi; *S*, sacculus; *U*, utriculus.

an apocrine type of mucopolysaccharide secretion not found elsewhere in epithelia of the ear.

In the parietal fossa below the dermis, the endolymphatic ducts are embedded in a loose, jellylike, mucoid, connective tissue that sometimes (often in *Carcharhinus menisorrah*) contains scattered melanophores. Two sheets of skeletal muscle, one on either side, originate in heavy collagenous tissue between the chondrocranium and dermis near the rim of the fossa, and are inserted into the walls of the ducts where they are joined. There is considerable variation between both species and individuals in the shape and position of each muscle sheet. In most specimens it consists of a single, fan-shaped muscle extending transversely from fossa rim to duct. In others, it is augmented by a small thin muscle running longitudinally in the fossa, parallel to the duct. In addition to these superficial muscles, sections of the duct in adults show smooth muscle fibers and elastic connective tissue fibers encircling the lumen wall.

Fenestrae

Posterior to the chondrocranial openings for the endolymphatic ducts there are two additional openings near the V-shaped base of the fossa depression, the "fenestrae ovali," leading into the otic capsules. The outer (medial) side of the fenestra, oriented at an angle of about 15° (*C. limbatus*) to about 30° (*C. menisorrah*) to the vertical, is covered with a tough membraneous sheet of dense connective and perichondrial tissue. The inner (distal) side of the fenestra that faces the lumen of the otic capsule is covered with a taut, tough membrane formed of the connective and epithelial tissue of the posterior vertical canal.

In sections through the fenestra of pups (Fig. 5) the space between the inner and

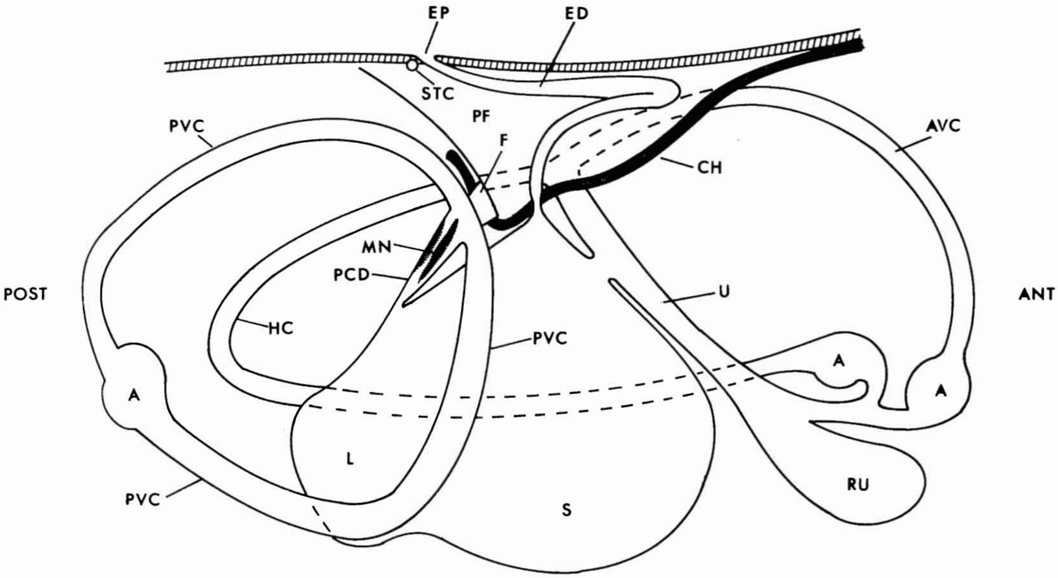

FIG. 3. Schematic diagram of the left ear of *Carcharhinus menisorrah* showing the relationship of the posterior canal duct to the fenestra, parietal fossa, sacculus, and endolymphatic duct. *A*, ampulla; *ANT*, anterior; *AVC*, anterior vertical canal; *CH*, chondrocranium (sectioned); *ED*, endolymphatic duct; *EP*, endolymphatic pore; *F*, fenestra; *HC*, horizontal canal; *L*, lagena; *MN*, macula neglecta; *PCD*, posterior canal duct; *PVD*, posterior vertical canal; *PF*, parietal fossa; *POST*, posterior; *RU*, recessus utriculi; *S*, sacculus; *STC*, supratemporal canal; *U*, utriculus.

outer membranes of the fenestra is filled with a very loose, mucoid, connective tissue, with a vascular plexus adjacent to the inner membrane. In gross dissection of fresh adult ears, however, the space is filled with liquid resembling the perilymph of the labyrinth. John F. Daniel (1928) described a similar double-layered "tympanum" in *Heptanchus* and reported that sandwiched between the two layers was a fluid that coagulated into a granular material in preserved specimens. This observation is omitted from the 1934 edition of his book. Coagulated material was not observed in preserved specimens of *Carcharhinus*.

Sacculus and Lagena

The sacculus is a triangular-shaped sac inclined at an angle of about 45° to the vertical (i.e., inclined in a ventrolateral direction to the middorsal line). It contains a large otolith composed of calcium carbonate granules embedded in a matrix of mucopolysaccharides. The granules are similar in shape and in size range to those of the endolymphatic duct. In fresh specimens the otolith has the consistency of thick cream. In preserved specimens, because of coagulation of the mucus, it forms a cohesive mass that can be removed intact or in pieces.

The bottom of the otolith rests on a pad of mucus in a troughlike, fibrous base forming the ventral wall of the sacculus. Nerve fibers enter this thickened base and branch several times before innervating the sensory epithelium of the macula sacculi that covers its inner surface. The macula consists of the usual barrel-shaped hair cells and elongated supporting cells, the latter presumably contributing to the pad of mucus between otolith and macula. Both the base and the medial wall of the sacculus are firmly attached to the chondrocranium.

The thin, lateral wall of the sacculus faces the lumen of the otic capsule. It is attached to a delicate network of connective tissue strands and capillaries that, along with the perilymph, fills the lumen. The epithelium of the lateral wall of the sacculus, above the macula, is convoluted into folds with numerous fingerlike villi. The columnar cells of the folds secrete mucopolysaccharides in a merocrine type of

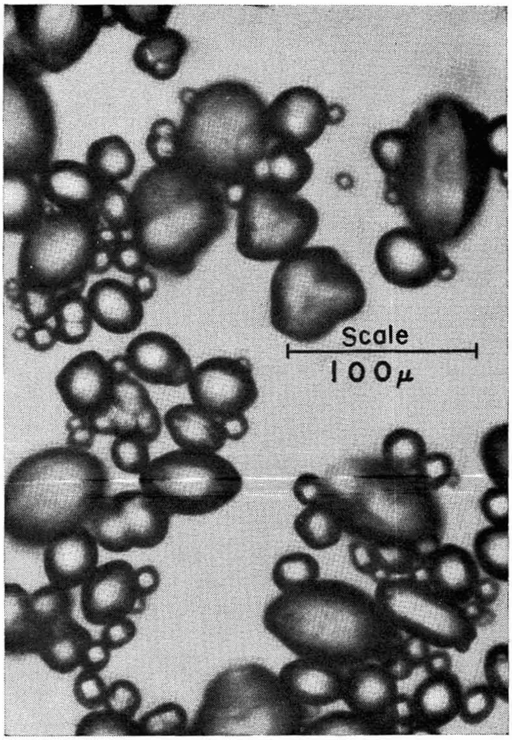

FIG. 4. Calcium carbonate granules from the endolymphatic duct of *Carcharhinus menisorrah.*

activity, presumably contributing to the otolith matrix. Between the folds, cuboid cells form a "vesiculated" epithelium similar to that described in the lateral line head canals of *Carcharhinus* adjacent to the neuromast (Tester and Kendall, 1969).

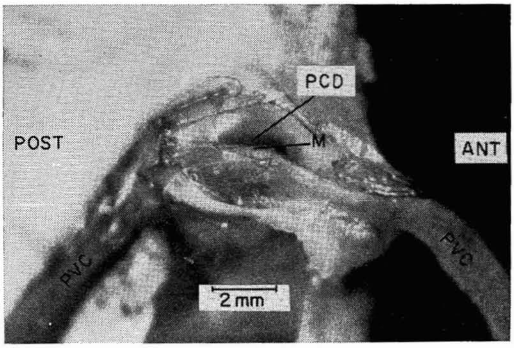

FIG. 5. Dissection of the right ear of *Carcharhinus menisorrah* showing the membrane (*M*) comprising the "roof" of the posterior vertical canal, slit open to expose the opening to the posterior canal duct (*PCD*).

The posterior ventral corner of the sacculus expands into a small pouch, the lagena, which has a patch of sensory epithelium on its fibrous, troughlike base. A protrusion of the sacculus otolith covers the macula lagenae.

About halfway along its posterior edge, the sacculus opens by a round pore into the posterior canal duct which, in turn, connects with the posterior vertical canal.

Utriculus and Semicircular Canals

The dorsal edge of the sacculus opens through a small slit into the thin-walled utriculus (called by some authors the anterior utriculus). The utriculus terminates anteriorly in a thickened, bulbous pouch, the recessus utriculi. On the floor of the pouch lies a patch of sensory epithelium, the macula utriculi, that is covered by a half-sphere of otolith material of similar composition to that of the sacculus.

The utriculus also receives the anterior vertical and the horizontal semicircular canals. Their ampullae contain saddle-shaped cristae oriented transversely to the canal plane. A single cupula, shrunken in sections, rests on the sensory epithelium that lines the saddle.

Posterior Canal Duct and Macula Neglecta

The posterior canal duct is a tapering, thin-walled tube that is fragile in fresh material but is somewhat tougher after preservation. It is roughly circular in cross section, of smallest bore (about 1 mm in a 160-cm specimen) where it connects to the posterior edge of the sacculus, and of largest bore (about 3 mm) where it connects to the posterior vertical canal (Fig. 3 and 5). Anteriorly, it is closely applied to the posterior edge of the sacculus over most of its length. Posteriorly, the lower half projects into the otic capsule of the sacculus but the upper half is nearly surrounded by and closely applied to a barrel-shaped cartilagenous sheath which also supports the posterior juncture of the horizontal and anterior vertical canals with the utriculus.

A longitudinal cut through the wall of the duct, with appropriate staining, reveals the macula neglecta that actually consists of two separate patches of sensory epithelium of unequal size. The larger of the two extends nearly the full length of the duct along the medial wall. The smaller, on the lateral wall, is narrower and shorter. Dorsad, it lies opposite the medial sensory epithelium but ventrad it twists one-fourth of a turn until it ends ventrally.

In freshly killed and immediately preserved *C. menisorrah* and *C. milberti* adult specimens, the duct may be completely filled with a single, sausage-shaped, coagulated cupula that can be removed intact. On the anterior face of the cupula is a shallow groove that expands considerably about halfway down its length. This groove lies against folds in the epithelial lining of the duct (see last paragraph of this section). Dorsally, in the preserved material, a tail of cupular material may extend into the lumen of the posterior canal. Ventrally, another tail may project through the opening to the sacculus. Under magnification, the cupula has a typical striated appearance, apparently consisting of many columns of mucus pressed together, as found in the lateral line neuromast cupulae of *Carcharhinus* by Tester and Kendall (1968, 1969). The surface appears brushy, presumably due to the breaking of mucus strand attachments to the epithelium.

In fresh-frozen (subsequently thawed) heads of juvenile and adult *Carcharhinus,* the duct is usually filled with a clear liquid. Presumably the delicate cupula disintegrated during the several hours between death and freezing. In several heads of *C. milberti* and *C. limbatus,* alternately frozen and thawed (due to freezer malfunction), the duct contained calcium carbonate granules which, on subsequent preservation in formalin, coagulated into a coherent mass resembling a sabre-shaped otolith. It is reasonably certain that this was an artifact for, in some specimens, the granules had even penetrated the otic chamber. It is probable that, in all species of the genus *Carcharhinus,* the sensory epithelia of the duct are intimately associated with a single, delicate, gelatinous cupula rather than with an otolith. However, de Burlet (1934) remarked that otoliths rather than cupulae occasionally are present in fishes, thus supporting his "neutral" designation of "papilla neglecta."

In sections of unborn pups of *C. menisorrah* the two separate patches of sensory epithelium are readily distinguished from the intervening nonsensory epithelium. They consist of the usual barrel-shaped hair cells embedded in elongated supporting cells that extend from a basement membrane to the distal surface. Below the basement membrane there is the usual thickened, highly vascularized layer of connective tissue containing nerve fibers and fibrillae. Although there are two separate patches of sensory epithelia, as in the adult, there is only a single cupula (cf. de Burlet, 1934). The cupula is usually shrunken in sections but still shows a groove where it was applied to folds in the epithelium (Fig. 6 and 7). It stains metachromatically with Giemsa, indicating a mucopolysaccharide composition, and shows typical striations interrupted distally from the epithelium by irregularly shaped cavities. In contrast, sections of the sacculus otolith

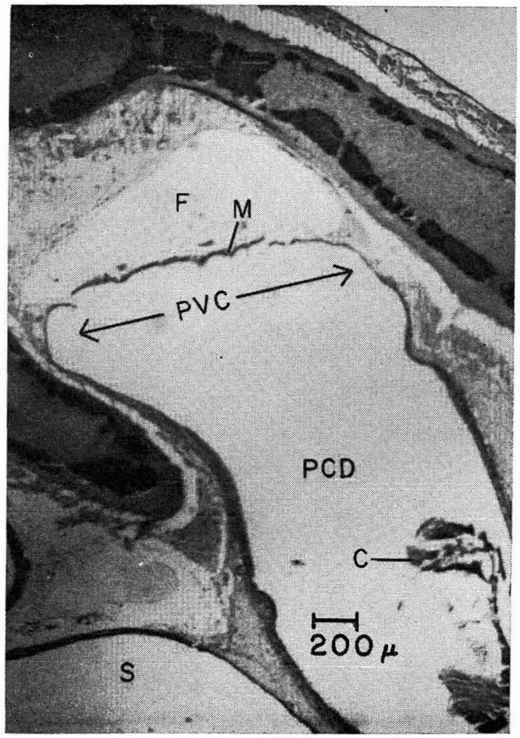

FIG. 6. Section of ear of *Carcharhinus menisorrah* pup, cut sagittally through the posterior canal duct (*PCD*) where it opens to the posterior vertical canal (*PVC*), showing the membrane (*M*) on the ventral side of the fenestra (*F*) and the shrunken cupula (*C*) within the posterior canal duct. A part of the sacculus (*S*) is seen at the lower left. Sections stained with hematoxylin and eosin.

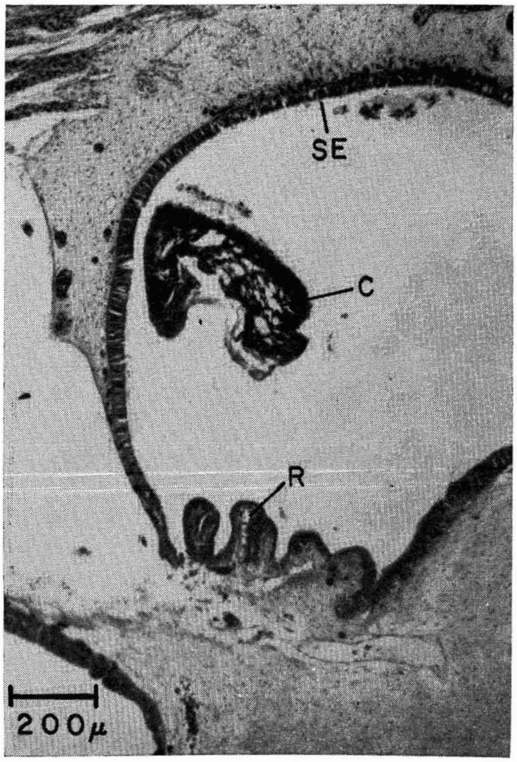

FIG. 7. Section across the posterior canal duct of the ear of *Carcharhinus menisorrah* showing one of the two patches of sensory epithelium (*SE*) of the macula neglecta, and the shrunken cupula (*C*) that is notched to fit over folds or ridges (*R*) comprised of vesiculated epithelial cells. Sections stained with Mallory's triple.

show football-shaped granules (or cavities, when decalcified) embedded in mucopolysaccharides from proximal to distal surface.

The nonsensory epithelial lining of the duct consists of columnar, mucus-secreting cells except on the anterior surface where folds are present (Fig. 7). In both pup and adult, the folds are considerably larger than those in the lateral wall of the sacculus. The cells of the folds and those adjacent to them form a "vesiculated" epithelium similar to that between the folds in the sacculus. Mucopolysaccharide granules are found on the distal surface of the folds but their origin is uncertain. It has been suggested that "vesiculated" epithelia such as these participate in ion exchange between capillaries and endolymph (Tester and Kendall, 1969).

Posterior Vertical Canal

The posterior canal duct joins the posterior vertical canal at the juncture of its two sections, one section with a large lumen (sometimes called the posterior utriculus) which runs ventrally and then posterolaterally to its ampulla, and the other section with a small lumen that runs from the ampulla dorsally in an anteromedial direction back to the juncture. As already described, the dorsal wall of the posterior canal immediately opposite the entrance to the duct is modified into a thin, tough membrane that is stretched across the inner surface of the fenestra (Fig. 6).

DISCUSSION

Lowenstein and Roberts (1951) show that in the ray (*Raja clavata*) the macula neglecta is lodged in the medial dorsal part of the

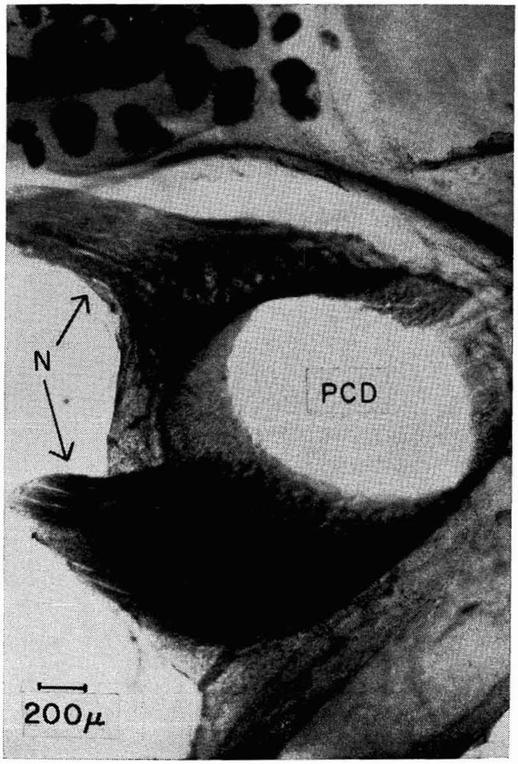

FIG. 8. Thick, frozen section of posterior canal duct (*PCD*) showing the two branches of ramus neglectus nerve (*N*) innervating the two patches of sensory epithelium of the macula neglecta. Sections impregnated with silver by Gilbert's technique.

sacculus cavity immediately posterior to the opening of the posterior canal duct that connects the sacculus with the full-circle ring of the posterior vertical canal. According to their diagram, the duct runs horizontally and joins the canal a considerable distance below the fenestra. They remarked that the position of the macula neglecta in the "immediate vicinity" of the fenestra, in conjunction with the external openings of the endolymphatic ducts situated nearby, "may well form part of a sound-conducting mechanism aiming at the sacculus in general and the macula neglecta in particular." They found that the macula neglecta was highly sensitive to vibration, "one of the most remarkable examples of sensitivity being a just noticeable response to the dropping of a pin from shoulder-height to the stone floor of the laboratory."

In sharks, the macula neglecta is even more strategically located with respect to the fenestra than it is in rays. In *Carcharhinus* (Fig. 3) it lies within the posterior canal duct which runs from the most dorsal segment of the posterior vertical canal to the sacculus in a posteroventrad-lateral direction. The opening of the duct to the posterior canal lies immediately opposite the fenestra. The fenestra is bounded by a taut, inner membrane formed by the roof of the canal and by a thicker, more flexible, outer membrane formed by the fibrous tissue lining the parietal fossa. In juvenile and adult, the chamber thus formed appears to be filled with liquid. Between the outer membrane of each fenestra and the integument, the fossa is filled with a mucoid connective tissue in which are embedded the recurrent endolymphatic ducts. The ducts usually contain calcium carbonate granules similar to those of the otoliths. A thin sheet of superficial muscle fibers runs from the juncture of the ducts to the connective tissue between the rim of the fossa and the dermis. The endolymphatic pores through the dermis are located above and slightly posterior to the fenestrae.

How could this system function in sound reception? If the chamber of the fenestra had contained an air bubble, it might have served as a transducer to convert sound pressure to particle velocity in much the same way as the swim bladder of teleosts. Since the chamber is full of liquid it might still serve to facilitate the passage of sound waves in that it offers less resistance than the dense, partly calcified cartilage of the chondrocranium. The macula neglecta seems well oriented for responding to displacements entering through the fenestra. The displacements could produce a shearing action on the cupula, thus activating the hair cells in the same manner as that postulated for the neuromasts of the lateral line canals (Dijkgraaf, 1963). In this regard it would be informative to determine, by electron microscopy, the orientation of the kinocilia with respect to the stereocilia for the hair cells of the shark macula neglecta, as has been done for the ray by Lowenstein, Osborne, and Wersäll (1964).

We can only speculate on how sound waves may reach the fenestra and how a system involving the macula neglecta may function. There are several possibilities, all subject to critical evaluation by a biophysicist versed in the complex field of sound transmission through tissues and liquids. (1) The dermis, which has elastic properties and is tightly stretched over the fossa, may vibrate in response to sound waves, producing and/or transmitting near field effects perhaps by virtue of some degree of compressibility of the mucoid connective tissue contained in the fossa. These displacements would then impinge on the elastic membrane of the fenestra. (2) The fossa, by virtue of its shape and dense walls, may act as an amplifier for the displacement waves thus produced. (3) The endolymphatic ducts, containing dense calcium carbonate granules and suspended laterally by muscles, may either amplify or produce displacement waves in the mucoid tissue when subject to displacement or pressure waves from an outside source. (4) The endolymphatic pores may serve to release displacement waves, thus enabling the system, consisting of essentially incompressible materials in the fenestra, posterior canal duct, sacculus, and endolymphatic duct, to function.

If the endolymphatic duct does not participate in the hearing mechanism, its function is puzzling. On first consideration it might seem that the duct could serve to equalize hydrostatic pressure in the ear with that in the water. However, this seems unlikely from pressure considerations of a chamber, such as the ear, filled with incompressible materials and immersed in water, even though open to the exterior by a duct.

The endolymphatic duct may serve as a storage bin for excess calcium carbonate granules produced in the sacculus or as a production site for granules to augment the supply in the sacculus. Since the granules in the duct are not associated with a sensory epithelium they serve no obvious *in situ* function.

Vilstrup (1951) suggested that in the dogfish (*Acanthias vulgaris*) calcium carbonate granules are generated in "vacuoles" of the endolymphatic duct epithelium and "pass down the lumen to the macular gelatinous substance in which they become embedded." In frozen sections of the duct of adult *Carcharinus* we did not see "vacuoles" containing granules in the cells of the wall, but we did see discrete groups of small granules in the lumen, each group encased in a mucous sheath, perhaps lending some support to his suggestions. Other evidence that particles may move from the duct to the sacculus comes from the dogfish in which, according to several authors and verified by us, the sacculus contains small sand grains which could only have entered via the endolymphatic pore and duct. Since in sections of the unborn pup of both *Acanthias* (Vilstrup, 1951) and *Carcharhinus,* well-formed calcium carbonate granules of the same shape and size range as in the adult are present in the sacculus but not in the endolymphatic duct, it may be that the duct is an auxiliary rather than a primary site of granule formation. From the size distribution and the occasional appearance of concentric layers in stained material, it appears that the granules are formed, and grow by accretion, not only in the duct but also in the sacculus and the recessus utriculi.

If the endolymphatic duct does not participate in the hearing mechanism, the function of the peculiar musculature system associated with the duct is also puzzling. This system consists not only of superficial skeletal muscle but also of concentric smooth muscle and elastic connective tissue fibers. Possibly it regulates either the influx of seawater or the efflux of endolymphatic material, or both. Another possibility is that the musculature system may move granules from the duct to the sacculus by a pumping action, similar to peristalsis.

Obviously there is a need for more information—behavioral, physiological, biophysical, and biochemical—on the elasmobranch ear before we can explain satisfactorily the functional significance of either the macula neglecta or the endolymphatic duct, both of which are primitive structures that have been reduced or lost during the evolution of the vertebrate ear.

ACKNOWLEDGMENTS

This study was supported in part by the Department of the Navy, Office of Naval Research, Contract Nonr 2756(00). Some of the material was supplied by Mr. Victor Faughnan, State Department of Land and Natural Resources, Division of Fish and Game, and by Dr. Richard C. Wass, former graduate student, Department of Zoology, University of Hawaii. We are indebted to Dr. Arthur N. Popper, also of the Department of Zoology, for fruitful discussions of how the shark ear may participate in hearing.

LITERATURE CITED

AYERS, H. 1892. Vertebrate cephalogenesis. J. Morph. 6:1–360.

BURLET, H.M. DE. 1934. Vergleichende Anatomie des stato-akustischen Organs. a. Die Innere Ohrsphäre, p. 1293–1432. *In* Bolk et al. [ed.] Handbuch der Vergleichenden Anatomie der Wirbeltiere. Vol. 2. Urban & Schwarzenberg, Munich.

CARLSTRÖM, D. 1963. A crystallographic study of vertebrate otoliths. Biol. Bull., Woods Hole **125**:441–463.

DANIEL, J. F. 1928. The elasmobranch fishes. Univ. Calif. Press, Berkeley. 332 p.

DAVENPORT, H. A. 1961. Histological and histochemical techniques. W. B. Saunders Co., Philadelphia and London. 401 p.

DAVIES, D. H., J. P. A. LOCHNER, and E. D. SMITH. 1963. Preliminary investigations on the hearing of sharks. S. Afr. Assoc. Mar. Biol. Res., Oceanogr. Res. Inst Investig. Rep. 7. 10 p.

DIJKGRAAF, S. 1963. The functioning and significance of the lateral-line organ. Biol. Rev. **38**:51–105.

HERZOG, H. 1925. Ueber die Entstehung der Otolithen. Z. Hals-Nas.-u. Ohrenheik. **12**: 413–422.

HOWES, G. B. 1883. On the presence of a tympanum in the genus *Raia*. J. Anat., Lond. **17**:188–190.

LOWENSTEIN, O., and T. D. M. ROBERTS. 1951. The localization and analysis of the responses to vibration from the isolated elasmobranch labyrinth. A contribution to the problem of the evolution of hearing in the vertebrates. J. Physiol. **114**:471–489.

LOWENSTEIN, O., M. P. OSBORNE, and J. WERSÄLL. 1964. Structure and innervation of the labyrinth of the thornback ray (*Raja clavata*). Proc. Roy. Soc., B. **160**:1–11.

QUIRING, D. P. 1930. Development of the ear of *Acanthias vulgaris*. J. Morph. **50**:259–294.

RETZIUS, G. 1881. Das Gehörorgan der Werbelthiere. I. Das Gehörorgan der Fische und Amphibien. Sansun & Wallin, Stockholm. 221 p.

———. 1884. Das Gehörorgan der Werbelthiere. II. Das Gehörorgan der Vögel und der Saugetiere. Sansun & Wallin, Stockholm. 368 p.

TESTER, A. L., and J. I. KENDALL. 1968. Cupulae in shark neuromasts: composition, origin, generation. Science **160**:772–774.

———. 1969. Morphology of the lateralis canal system in the shark genus *Carcharhinus*. Pacif. Sci. **23**:1–16.

VAN BERGEIJK, W. A. 1967. The evolution of vertebrate hearing, p. 1–49. *In* W. D. Neff [ed.] Contributions to sensory physiology. Vol. 2. Academic Press, New York and London.

VILSTRUP, T. 1951. On the formation of Otoliths. Ann. Otol., Rhinol., Laryngol., St. Louis **60**:974–981.